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Hyperhomocysteinemia Increases Risk of Death, Especially in Type 2 Diabetes

5-Year Follow-Up of the Hoorn Study

Ellen K. Hoogeveen, MD, PhD; Pieter J. Kostense, PhD; Cornelis Jakobs, PhD; Jacqueline M. Dekker, PhD; Giel Nijpels, MD, PhD; Robert J. Heine, MD, PhD; Lex M. Bouter, PhD; Coen D.A. Stehouwer, MD, PhD

Background—A high serum total homocysteine (tHcy) concentration is a risk factor for death, but the strength of the relation in patients with type 2 (non–insulin-dependent) diabetes mellitus compared with nondiabetic subjects is not known. A cross-sectional study suggested that the association between tHcy and cardiovascular disease is stronger in diabetic than in nondiabetic subjects. We therefore prospectively investigated the combined effect of hyperhomocysteinemia and type 2 diabetes on mortality.

Methods and Results—Between October 1, 1989, and December 31, 1991, serum was saved from 2484 men and women, 50 to 75 years of age, who were randomly selected from the town of Hoorn, The Netherlands. Fasting serum tHcy concentration was measured in 171 subjects who died (cases; 76 of cardiovascular disease) and in a stratified random sample of 640 survivors (control subjects). Mortality risks were calculated over 5 years of follow-up by means of logistic regression. The prevalence of hyperhomocysteinemia (tHcy >14 μmol/L) was 25.8%. After adjustment for major cardiovascular risk factors, serum albumin, and HbA_{1c}, the odds ratio (95% CI) for 5-year mortality was 1.56 (1.07 to 2.30) for hyperhomocysteinemia and 1.26 (1.02 to 1.55) per 5-μmol/L increment of tHcy. The odds ratio for 5-year mortality for hyperhomocysteinemia was 1.34 (0.87 to 2.06) in nondiabetic subjects and 2.51 (1.07 to 5.91) in diabetic subjects ($P=0.08$ for interaction).

Conclusions—Hyperhomocysteinemia is related to 5-year mortality independent of other major risk factors and appears to be a stronger (1.9-fold) risk factor for mortality in type 2 diabetic patients than in nondiabetic subjects. **(*Circulation*. 2000;101:1506-1511.)**

Key Words: mortality ■ cardiovascular diseases ■ diabetes mellitus ■ epidemiology

Cardiovascular disease is the major cause of death in diabetic and nondiabetic subjects. The overall and cardiovascular mortality rates are 2 to 4 times higher in type 2 diabetic patients than in nondiabetic subjects.[1–5] Type 2 diabetes is known to be associated with several other cardiovascular risk factors, including dyslipidemia and hypertension, but these do not fully explain the excess mortality rates in type 2 diabetes. Therefore, increased risk must be due, at least in part, to diabetes itself, poor metabolic control, or other factors.

Hyperhomocysteinemia is a recently recognized risk factor for cardiovascular disease that is independent of major risk factors such as diabetes, hypertension, hypercholesterolemia, and smoking.[6–9] The prevalence estimates of hyperhomocysteinemia (>14 μmol/L) vary between 5% and 30% in the general population.[10–13] Although the mechanisms by which homocysteine promotes atherothrombosis are unknown, the epidemiological evidence for the association of hyperhomocysteinemia with atherothrombotic disease is strong.[6,7,14] A meta-analysis[15] showed that treatment with 0.5 to 5.0 mg folic acid daily can lower serum total homocysteine (tHcy) by 15% to 40% within ≈6 weeks. In addition, it has been estimated that lowering tHcy by 5 μmol/L (≈1 SD) may reduce the risk of cardiovascular death by ≈10%.[7] Taken together, hyperhomocysteinemia may be an important modifiable risk factor, although this must be confirmed in randomized studies of homocysteine-lowering treatment.

In a cross-sectional analysis, hyperhomocysteinemia appeared to be a stronger risk factor for cardiovascular disease in type 2 diabetic subjects than in nondiabetic subjects.[13] Such an interaction between hyperhomocysteinemia and type 2 diabetes with regard to cardiovascular risk may be clinically

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From the Institute for Research in Extramural Medicine (E.K.H., J.M.D., G.N., L.M.B.) and the Department of Epidemiology and Biostatistics (P.J.K.), Vrije Universiteit, Amsterdam; and the Department of Clinical Chemistry (C.J.) and the Department of Internal Medicine (R.J.H., C.D.A.S.), University Hospital Vrije Universiteit, Amsterdam, The Netherlands.

Correspondence to Ellen K. Hoogeveen, MD, PhD, Institute for Research in Extramural Medicine, Vrije Universiteit, van der Boechorststraat 7, 1081 BT Amsterdam, The Netherlands. E-mail ellenhgv@casema.net

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important, as it implies that homocysteine-lowering treatment may be especially effective in type 2 diabetes. In view of these considerations, we investigated the combined effect of hyperhomocysteinemia and diabetes with respect to 5-year risk of death in a population-based study.

Methods

Design and Study Population

The Hoorn Study is a prospective study of glucose tolerance and other cardiovascular risk factors in a 50- to 75-year-old general white population. The baseline examination was conducted from October 1, 1989, until December 31, 1991.[16] Briefly, a random sample of all men and women 50 to 75 years of age was drawn from the municipal population registry office of Hoorn, The Netherlands; 2484 subjects were enrolled in this cohort (response rate 71%). All subjects, except previously diagnosed diabetic subjects treated with oral glucose-lowering agents or insulin, underwent a 75-g oral glucose tolerance test (OGTT) and were classified according to the World Health Organization (1985) criteria.[17] A second OGTT (participation rate 93%) was performed for reasons of efficiency in a random subsample (n=1122) stratified by 2-hour glucose values of the first test, age, and sex. Finally, from this subsample, an age-stratified, sex-stratified, and glucose tolerance–stratified random subsample (n=715), the "subcohort," was drawn. Glucose tolerance was divided into 3 categories on the basis of the mean of the 2 OGTTs: NGT, (n=334), IGT, (n=197), and type 2 diabetes mellitus (n=184).

A case-control study nested within the cohort was carried out. The survivors of the subcohort (as defined above) served as control subjects. Every subject who died within 5 years of follow-up of the entire cohort was ascertained and selected for the present study. Information on subjects' vital status on January 1, 1997, was collected from the mortality registry of the municipality of Hoorn. Information on vital status of 137 subjects who moved out of town was obtained from the new local municipalities. We determined whether each subject had died during or survived the first 5 years of follow-up. Causes of death were extracted from medical records of the general practitioners and the hospital of Hoorn, verified by a physician, and classified according to the 9th revision of the International Classification of Diseases (ICD).[18] Death from cardiovascular disease was defined by ICD codes 390-459.

During the 5-year follow-up, 172 participants died, 75 of whom were included in the subcohort (n=715). No serum was available for the measurement of tHcy from 1 of the subjects who died. Thus, analyses were performed on 811 subjects, and tHcy was measured in stored sera. The Hoorn Study was approved by the Ethics Review Committee of the University Hospital Vrije Universiteit Amsterdam. Informed consent was obtained from all participants.

Measurement of tHcy

Fasting blood samples were centrifuged within 1 hour after collection. Serum was stored at −20°C for 4 to 7 years. There is good evidence that serum tHcy concentrations are stable for ≥10 years.[19] Serum total (free plus protein bound) homocysteine was measured with tri-*n*-butylphosphine as the reducing agent and ammonium 7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate as the thiol-specific fluorochromophore, followed by high-performance liquid chromatography with fluorescence detection.[20] The intra-assay and interassay coefficients were 2.1% and 5.1%.

Other Variables

Blood pressure was measured as the mean of 4 measurements taken on 2 different occasions with the use of a random-zero sphygmomanometer under standardized conditions. Fasting and 2-hour postload venous plasma glucose concentrations were measured with a glucose dehydrogenase method (Merck). Fasting serum total cholesterol, HDL-cholesterol, and triglycerides were measured by enzymatic techniques (Boehringer-Mannheim). Serum albumin was assessed with the bromcresol purple method. Hypoalbuminemia was defined as albumin ≤34 g/L.[21] All laboratory measurements were carried out in a blinded fashion with respect to mortality, glucose tolerance status, and other clinical data.

Statistical Analysis

Prevalence of hyperhomocysteinemia, defined as serum tHcy level >14 μmol/L,[12] in the entire cohort was back-calculated by means of direct standardization. Briefly, the prevalence of hyperhomocysteinemia was determined in 24 strata [age (50 to 59, 60 to 69, and 70 to 75 years), sex (male and female), and glucose tolerance (NGT, IGT, and newly diagnosed and known type 2 diabetes mellitus)] of the subsample. To assess the prevalence of hyperhomocysteinemia in the original population-based sample (standard n=2484), the prevalence of hyperhomocysteinemia was back-calculated from the magnitude of each age, sex, and glucose tolerance category stratum.

We assessed the relation between tHcy and 5-year overall mortality in the nested case-control study with logistic regression analyses. We calculated odds ratios plus 95% CI for serum tHcy both as a continuous variable, expressed per 5-μmol/L (≈1 SD) increment of serum tHcy, and as a categorical variable [divided in 2 (>14 μmol/L vs ≤14 μmol/L) and in 4 categories (≤9.0 μmol/L, 9.1 to 14.0 μmol/L, 14.1 to 19.0 μmol/L and >19.0 μmol/L)]. Odds ratios of mortality were adjusted for the stratifying variables (ie, age, sex, and glucose tolerance) and potentially confounding major cardiovascular risk factors (ie, hypertension, hypercholesterolemia, and current smoking[11,22]). Possible interactions between tHcy and cardiovascular risk factors were assessed in stratified analyses and with interaction terms by means of logistic regression.

To assess whether the observations were distorted by underlying disease that might cause both high values of serum tHcy and increased mortality rates,[23] we did 2 additional analyses. First, we adjusted for serum albumin, an acute-phase protein and a putative marker of health and nutrition status.[24] Second, we adjusted for the presence of cardiovascular disease at baseline, as defined elsewhere,[13] although the latter analysis might obscure a true effect because cardiovascular disease may well be an intermediate factor in the causal pathway linking tHcy to mortality.[25]

Finally, we assessed the relation between tHcy and cardiovascular death over the first 5-years of follow-up. This analysis was restricted to the subcohort because it required the Cox proportional hazards model. All analyses were performed with SPSS for Windows 95 version 7.5.2.

Results

The baseline characteristics of the patients who died and the control subjects are presented in Table 1. The back-calculated prevalence of hyperhomocysteinemia (>14 μmol/L) in the cohort was 25.8%. Of all type 2 diabetic subjects, 115 (62.5%) were newly diagnosed and 69 (37.5%) were known to have diabetes and were treated with glucose-lowering agents: 16 (8.7%) with insulin, 52 (28.3%) with sulfonylureas, and 3 (1.6%) with metformin (of whom 2 also used sulfonylureas). The median known duration of diabetes of subjects in whom type 2 diabetes had previously been diagnosed was 6.4 years (interquartile range 2.7 to 12.0). Serum tHcy concentrations did not correlate with fasting glucose (r=0.001; P=1.0), HbA_{1c} (r=−0.03; P=0.4), or serum albumin (r=−0.03; P=1.0). The mean serum tHcy concentration in diabetic subjects treated with insulin or glucose-lowering agents was 12.3 μmol/L (SD 8.6 μmol/L) versus 12.5 μmol/L (SD 4.6 μmol/L) in those not so treated (P=0.1); it was 12.3 μmol/L (SD 4.2 μmol/L) in subjects treated with insulin and 12.3 μmol/L (SD 9.6 μmol/L) in those treated with glucose-lowering agents (P=0.4).

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TABLE 1. Baseline Characteristics of the Study Population

	Cases†	Control Subjects‡
n	171	640
Men,* %	58.5	46.4
Age,* y	66.6 (7.1)	63.9 (7.0)
Body mass index, kg/m^2	26.7 (4.2)	27.2 (4.0)
Cigarette smokers, current, %	41.4	27.9
Systolic blood pressure, mm Hg	141 (23)	139 (19)
Diastolic blood pressure, mm Hg	82 (12)	82 (10)
Hypertension,§ %	50.3	37.3
Impaired glucose tolerance,* %	10.5	28.1
Diabetes mellitus,* %	22.2	22.8
Plasma fasting glucose, mmol/L	6.6 (2.9)	6.5 (2.2)
HbA_{1c}, % of hemoglobin	6.0 (1.4)	5.8 (1.2)
Serum total cholesterol, mmol/L	6.7 (1.1)	6.7 (1.2)
Hypercholesterolemia,‖ %	61.4	54.1
Serum HDL cholesterol, mmol/L	1.2 (0.4)	1.3 (0.4)
Total:HDL cholesterol ratio	5.9 (1.8)	5.5 (1.7)
Serum triglycerides, mmol/L	1.6 (1.2-2.2)	1.5 (1.1-2.1)
Dyslipidemia,¶ %	42.7	33.0
Serum albumin, g/L	38 (3.2)	39 (2.9)
Serum total homocysteine, μmol/L	12.9 (9.9-16.2)	11.5 (9.4-14.1)

Data are presented as mean (SD), percentage of the total, or median (interquartile range).

*Stratifying variable.

†Cases are subjects who died during the 5-year follow-up.

‡Control subjects are survivors taken from an age-stratified, sex-stratified, and glucose tolerance–stratified random sample of the cohort (see Methods).

§Hypertension was defined as blood pressure ≥160 mm Hg systolic and/or ≥95 mm Hg diastolic and/or current use of antihypertensive medication.

‖Hypercholesterolemia was defined as total cholesterol ≥6.5 mmol/L and/or current use of cholesterol-lowering medication.

¶Dyslipidemia was defined as levels of triglycerides >2.3 mmol/L and/or levels of HDL cholesterol <1.0 mmol/L in men and <1.1 mmol/L in women.

Overall Mortality

The cause of death could be found in 93.6% (160 of 171); 47.5% (76 of 160) died of cardiovascular disease, of whom 34 belonged to the subcohort. The 84 (52.5%) noncardiovascular deaths were due to malignant neoplasms (60), septicemia (3), respiratory tract infection (3), respiratory disease (1), external causes (3), and other causes (14).

In the entire cohort, the 5-year risk of death was 5.7% in subjects with normal glucose tolerance (NGT), 7.1% in subjects with impaired glucose tolerance (IGT), and 18.5% in subjects with diabetes; it was 5.5% in subjects with serum tHcy ≤14 μmol/L and 10.8% in subjects with serum tHcy >14 μmol/L.

The risk of 5-year overall mortality increased considerably above a serum tHcy concentration of 14 μmol/L (Figure 1). Table 2 shows the odds ratios of overall mortality in the presence versus the absence of other major cardiovascular risk factors. Additional adjustment for dyslipidemia, body mass index, or pack-years of smoking did not attenuate the strength of the association between serum tHcy and death, nor did additional adjustment for serum albumin (Table 2). There was a graded inverse relation between serum albumin and death that was not altered by adjustment for potential confounders (Table 2). Subjects with hypoalbuminemia had a 2.2-fold (95% CI 1.2 to 3.9) greater risk of death compared with subjects with serum albumin levels >34 g/L.

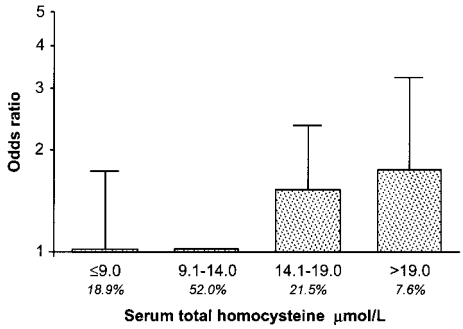

Figure 1. Odds ratios for 5-year overall death according to serum tHcy level adjusted for age, sex, diabetes, hypertension, hypercholesterolemia, current smoking, serum albumin, and HbA_{1c}. Reference category was serum tHcy values 9.1 to 14.0 μmol/L. Percentages of population under study for each serum tHcy range are shown. Error bars represent upper half of 95% CI (P=0.04 for trend).

We evaluated possible interaction and did not observe substantial differences among the strata of the following risk factors: male sex, hypertension, hypercholesterolemia, and current smoking (data not shown). After stratification by diabetes and adjustment for age, sex, hypertension, current smoking, hypercholesterolemia, and serum albumin in the logistic regression model, the odds ratio of 5-year mortality associated with hyperhomocysteinemia was, however, 1.34 (0.87 to 2.06) in nondiabetic subjects and 2.51 (1.07 to 5.91) in diabetic subjects (P=0.08 for interaction; Figures 2 and 3). This indicates that hyperhomocysteinemia is a stronger (1.9-fold, 95% CI 0.7 to 4.9) risk factor for death in diabetic than in nondiabetic subjects. For each 5-μmol/L increment of serum tHcy, the odds ratio was 1.17 (0.92 to 1.50) in nondiabetic subjects and 1.60 (1.02 to 2.51) in diabetic subjects. (Subjects with NGT and IGT were pooled because the odds ratio of 5-year mortality associated with hyperhomocysteinemia did not differ substantially between these categories and the odds ratio remained similar if NGT and IGT were pooled; data not shown.) An additional analysis revealed that among diabetic subjects with hyperhomocysteinemia, those with known diabetes had the highest relative risk of mortality. After adjustment for age and sex, the odds ratio of 5-year mortality associated with hyperhomocysteinemia was 2.58 (0.90 to 7.40) for subjects with newly diagnosed diabetes and 3.18 (0.74 to 13.74) for subjects with known diabetes. This interaction showed a significant trend (P=0.04): The odds ratio increased gradually over the 3 subgroups: nondiabetic, newly diagnosed diabetic, and known diabetic subjects. Finally, in a stratified analysis, after adjustment for cardiovascular risk factors and the presence of cardiovascular disease at baseline, we again found interaction: The odds ratio of 5-year mortality associated with hyperhomocysteinemia was 1.27 (0.82 to 1.96) in nondiabetic subjects and 2.55 (1.08 to 6.02) in diabetic subjects (P=0.07 for interaction).

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TABLE 2. Odds Ratios (95% CI) for 5-Year Overall Mortality

Risk Factors	Adjusted for Age, Sex, and Diabetes	Adjusted for Age, Sex, Diabetes, and Other Risk Factors*
Hyperhomocysteinemia (>14 vs ≤14 μmol/L)	1.58 (1.09–2.28)†	1.56 (1.07–2.30)
Total homocysteine (per category increment)‡	1.31 (1.06–1.63)	1.27 (1.02–1.59)
Total homocysteine (per 5-μmol/L increment)§	1.31 (1.06–1.60)	1.26 (1.02–1.55)
Hypertension (yes/no)	1.60 (1.12–2.28)	1.58 (1.08–2.29)
Current smoking (yes/no)	2.01 (1.39–2.90)	1.66 (1.13–2.45)
Hypercholesterolemia (yes/no)‖	1.49 (1.04–2.13)	1.45 (1.00–2.11)
Serum albumin (per 2.5-g/L increment)	0.72 (0.62–0.84)	0.73 (0.63–0.86)
HbA_{1c} (per % increment)	1.24 (1.05–1.48)	1.17 (0.98–1.40)

*Adjusted for age, sex, diabetes, and the other 5 risk factors mentioned in this table. When these analyses were adjusted for homocysteine, homocysteine was entered as a 4-category variable in the models.

†After additional adjustment for dyslipidemia: 1.58 (1.09 to 2.29) or for body mass index: 1.54 (1.06 to 2.23).

‡Serum total homocysteine was divided into 4 categories (see Methods).

§After exclusion of 8 outliers (1 case and 7 control subjects with serum total homocysteine >35 μmol/L); if outliers were included, the odds ratios were 1.10 (0.96 to 1.25) and 1.10 (0.95 to 1.26).

‖If hypercholesterolemia was replaced by total:HDL cholesterol ratio, odds ratios were 1.18 (1.07 to 1.31) and 1.12 (1.01 to 1.24).

Cardiovascular Death

The mean serum tHcy concentration did not differ between subjects who died of cardiovascular and noncardiovascular causes (13.9 μmol/L, SD 6.5 μmol/L, and 13.5 μmol/L, SD 5.0 μmol/L; $P=0.6$) but was higher in subjects who died of cardiovascular disease compared with those who survived the first 5 years of follow-up (13.9 μmol/L, SD 6.5 μmol/L, and 12.6, SD 5.9 μmol/L; $P=0.006$).

After adjustment for the stratifying variables, the hazard ratio (95% CI) of cardiovascular death was 1.65 (0.81 to 3.31) for hyperhomocysteinemia, 1.58 (1.04 to 2.42) for each category increment of serum tHcy, and 1.55 (1.08 to 2.23) for each 5-μmol/L increment of serum tHcy. After additional adjustment for hypertension, hypercholesterolemia, and current smoking, these hazard ratios were 1.60 (0.65 to 3.01), 1.51 (0.98 to 2.32), and 1.45 (1.01 to 2.08), respectively. Because of the limited number of cases in the subcohort, we could not investigate the issue of interaction of hyperhomocysteinemia and diabetes with regard to cardiovascular death.

Discussion

This prospective, population-based study, with a 5-year follow-up, indicates that hyperhomocysteinemia is a risk factor for overall mortality in type 2 diabetic patients, independent of major cardiovascular risk factors and serum albumin, a putative general marker of health. Moreover, hyperhomocysteinemia appeared to be a stronger (≈2-fold) risk factor for death in diabetic than in nondiabetic subjects. For each 5-μmol/L (≈1 SD) increment of serum tHcy, the risk of 5-year mortality rose by 17% in the nondiabetic and by 60% in the diabetic subjects.

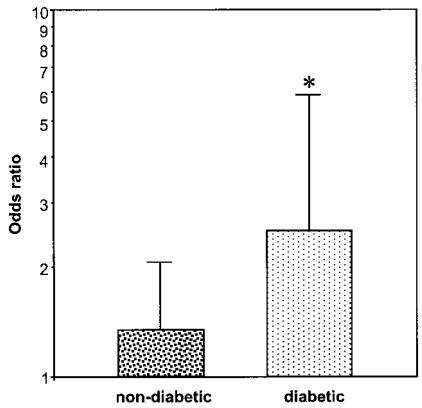

Figure 2. Odds ratios for 5-year overall death associated with hyperhomocysteinemia (>14 μmol/L) after stratification by diabetes (yes/no). Error bars represent upper half of 95% CI. Odds ratios are adjusted for age, sex, hypertension, hypercholesterolemia, current smoking, and serum albumin (*$P<0.05$, $P=0.08$ for interaction).

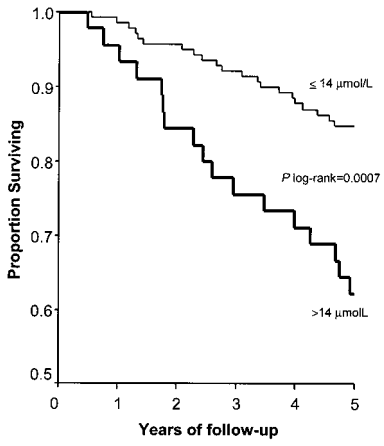

Figure 3. Estimated survival among type 2 diabetic subjects in subcohort (see Methods), according to presence of hyperhomocysteinemia (yes/no). Survival was estimated with Kaplan-Meier product-limit method compared with log-rank test.

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There are several prospective studies that have investigated the relation between tHcy and risk of cardiovascular disease. Many[9,26–36] but not all[23,37,38] found a positive relation. None of the previous studies, however, investigated the possibility of interaction between hyperhomocysteinemia and diabetes with regard to risk of death. The design of the present study, with a high prevalence and an accurate diagnosis of type 2 diabetes, provided an opportunity to do so. The strength of the relation between hyperhomocysteinemia and death appeared to be stronger among those with diabetes than among those without diabetes. An interaction of hyperhomocysteinemia with diabetes is biologically plausible. High homocysteine concentrations may exert an atherothrombotic effect through increasing oxidative stress, which may induce endothelial dysfunction.[14,39–41] Homocysteine can also affect the properties of the extracellular matrix and increase smooth muscle cell proliferation.[14] Oxidative stress is thought to be increased in type 2 diabetes,[42] and matrix alterations are a prominent feature of diabetes in general, both of which might make diabetes patients more susceptible to the adverse affect of hyperhomocysteinemia. The interaction with hyperhomocysteinemia observed in the present study, if confirmed, may have important implications with regard to risk management in type 2 diabetes.

Little is known about the impact of diabetes per se or its treatment on tHcy metabolism.[13,43] In the present study, we found no relation between tHcy and fasting glucose or HbA_{1c}. However, ≈40% of the diabetic subjects had previously been diagnosed, and we therefore cannot rule out that changes of dietary habits may have resulted in an increase of vitamin B intake.

The present study has several limitations. (1) We lacked data on intake and serum levels of folate, vitamin B_{12}, and vitamin B_6. We therefore were unable to explore the extent to which the relation between B vitamins and serum tHcy levels differs for diabetic and nondiabetic subjects. It has been suggested that hyperglycemia may cause an increased loss of water-soluble B vitamins.[44] On the other hand, relative renal hyperfiltration among diabetic subjects may result in lower tHcy levels.[45] (2) As in any study, our data were subject to classification errors. Errors in coding cause of death would not affect our analyses of overall mortality, but they would affect the count of deaths from specific causes such as cardiovascular deaths. Such errors are not likely to be related to tHcy assessment, however, and therefore will result in nondifferential misclassification, tending to underestimate the strength of the relation between hyperhomocysteinemia and cardiovascular death.[25] In addition, owing to small numbers, we could not assess the effect of hyperhomocysteinemia for cardiovascular death as precisely as for overall mortality. (3) Because the follow-up period was relatively short (5 years), we cannot exclude the possibility that tHcy levels are elevated because of the presence of (sub)clinical atherosclerosis.[23,46] However, adjustment for cardiovascular disease at baseline or serum albumin did not attenuate the relation between hyperhomocysteinemia and mortality. (4) Finally, we cannot rule out the possibility that incomplete adjustment for some cardiovascular risk factors may have resulted in residual confounding.

In conclusion, this study indicates that hyperhomocysteinemia is a risk factor for overall mortality and for cardiovascular death during a 5-year follow-up. The effect does not appear to be explained by other major cardiovascular risk factors. It is likely to be a stronger risk factor for overall mortality in diabetic patients than among nondiabetic subjects. Nevertheless, although strong evidence from this and other studies has accumulated linking hyperhomocysteinemia to cardiovascular disease, persuasive inferences about a causal role will likely emerge only from large randomized trials in which subjects are allocated to either homocysteine-lowering therapy or standard preventive approaches.

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